

Fig.1 Evaluation of different solvents to extract cocoa proteinaceous material. The per cent of solvent indicated is by vol. Unless indicated otherwise each extraction was carried out at concentrations of 10 % (w/v). Total amino groups and amino acids in the acid hydrolyzed cocoa nib powder were assumed to be maximum extractable amounts. (■) N_t, total amino groups and (▨) A_t, total amino acids. For details see "Experimental Procedures". CPB3, citrate phosphate buffer (50 mM; pH 3), CPB8, citrate phosphate buffer (50 mM; pH 8), PB8/NaCl, 50 mM sodium phosphate buffer, pH 8 plus 0.5 M NaCl, MeOH, methanol, PB7, 10 mM potassium phosphate buffer, pH 7.0, ISP, isopropanol, FA, formic acid, AcA, acetic acid.

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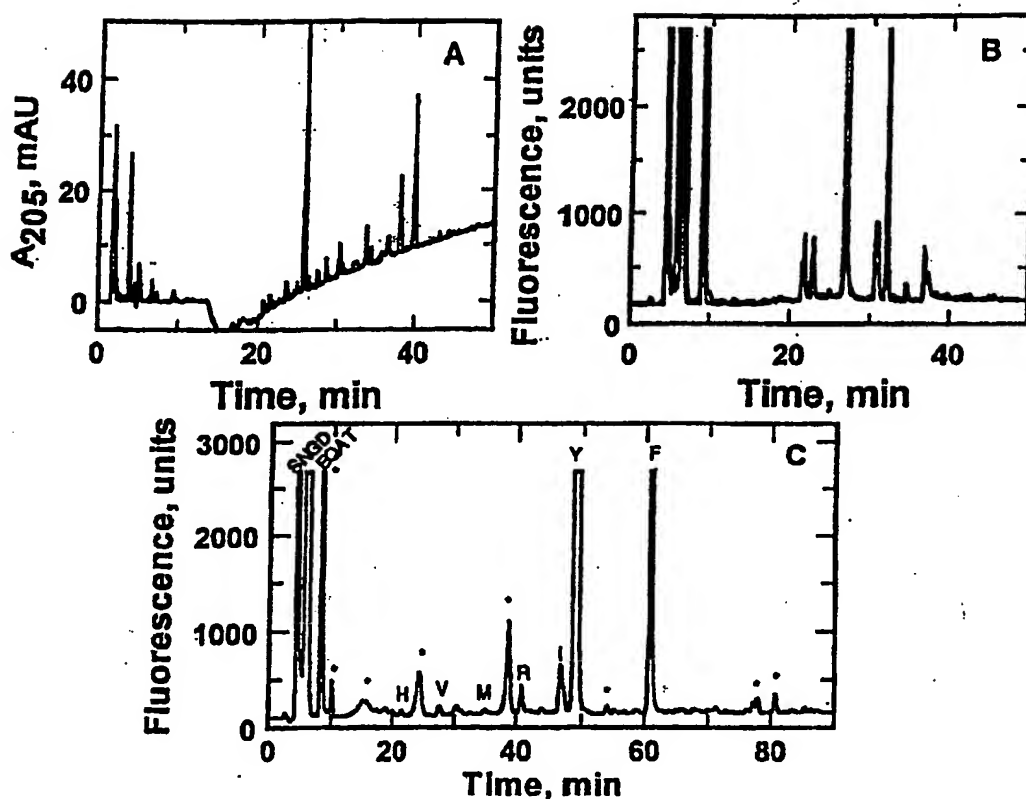


Fig. 2 RP-HPLC of SPE fractions. Appropriately diluted samples were injected onto RP column (Spherisorb 80-5C8 (250 x 4.6 mm)) and eluted with TFA/HSA/ACN solvent system (see Experimental Procedure for) with gradient isocratic at 0 % B for 10 min, 0-50 % B in 60 min, isocratic at 40 % B for 5 min, 50-100 % B in 25 min and isocratic at 100 % B for 5 min. The peaks were detected by UV absorbance at 205 nm (A) and by fluorometric detection (B) following post-column reaction with OPA reagent. C, Optimized gradient (0 % B for 20 min, 0-25 % B in 70 min, 25 to 100 % B in 10 min and isocratic elution at 100 % B for 10 min. Red trace, fermented Aca extract and blue trace, unfermented Aca extract